
بسم الله الرحمن الرحيم

# Biotechnology

Section 3

# Objectives

- Macroscopic examination of *Streptomyces*.
- Microscopic examination.
- Streaking for isolation.

# Macroscopic and Microscopic characters of Streptomyces

- *Streptomyces* are Gram positive, spore-forming bacteria found in soil.
- They are characterized by their tough, leathery, frequently pigmented colonies and their filamentous growth.
- *Streptomyces* are chemoheteroorganotrophs, growing best at 25°C and pH 8-9.
- They use complex organic materials as carbon and energy sources and are involved in the breakdown of these products in the soil.
- This degradative ability makes these bacteria pivotal in the production of fertile soil for agriculture.

- They also give soil its characteristic **smell** by the production of volatile low molecular weight compounds called **geosmins**.
- *Streptomycetes* are also of medical and industrial importance because they synthesize **antibiotics**.
- Those antibiotics help the organism compete with other organisms in the nutrient-depleted environment of the soil by reducing competition.
- Over 50 different antibiotics have been isolated from *Streptomycetes* species, including **streptomycin**, **neomycin**, **chloramphenicol** and **tetracyclines**.

# Identification and Examination of soil micro-organisms

**Materials**

- 1-2 media plates
- wire loop
- Bunsen burner

***Streptomyces*** **species are white and colorful chalky looking colonies.**

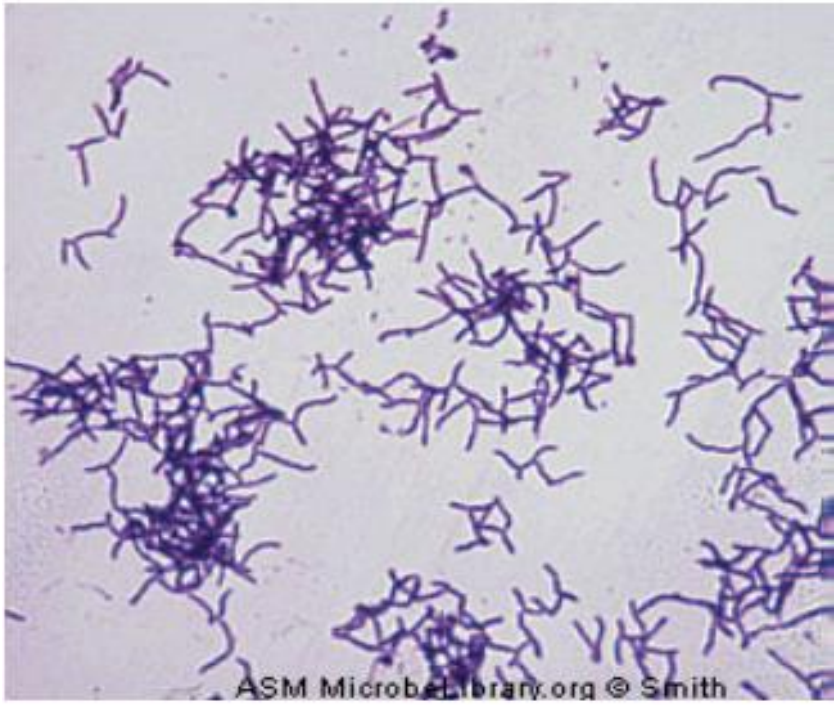
# 1-Macroscopic examination

- Examine the ISP2 plates and look for typical *Streptomyces* colonies.
- They are small, opaque, compact, frequently pigmented (brown, yellow, pink, etc.), often leathery, and appear dry and dull looking.
- Typically, a depression in the agar surface will be observed around the colony.
- Avoid molds. They usually form much softer, fuzzy colonies if present.

- Good *Streptomyces* candidates will be **difficult to remove from the agar with the inoculating needle or loop** and upon observation under the microscope will reveal a multitude of spores with a few filamentous cells.

# 2- Staining of *Sterptomyces* by Gram stain

- Using a flamed loop, scrape part of a **colony** onto a drop of **water** on a **slide**. Let dry.
- Heat fix by gently passing the slide through the flame 3 or 4 times.
- Flood slide with **crystal violet** for 15 secs, wash with water.
- Flood with **iodine** for 1 min, wash.
- Add 2 drops of **95% ethanol** for 5 seconds, wash.
- Flood with **safranin** for 5 min, wash.
- Dry the slide, add 1 drop of ceder wood oil.
- View using oil immersion lens (100X).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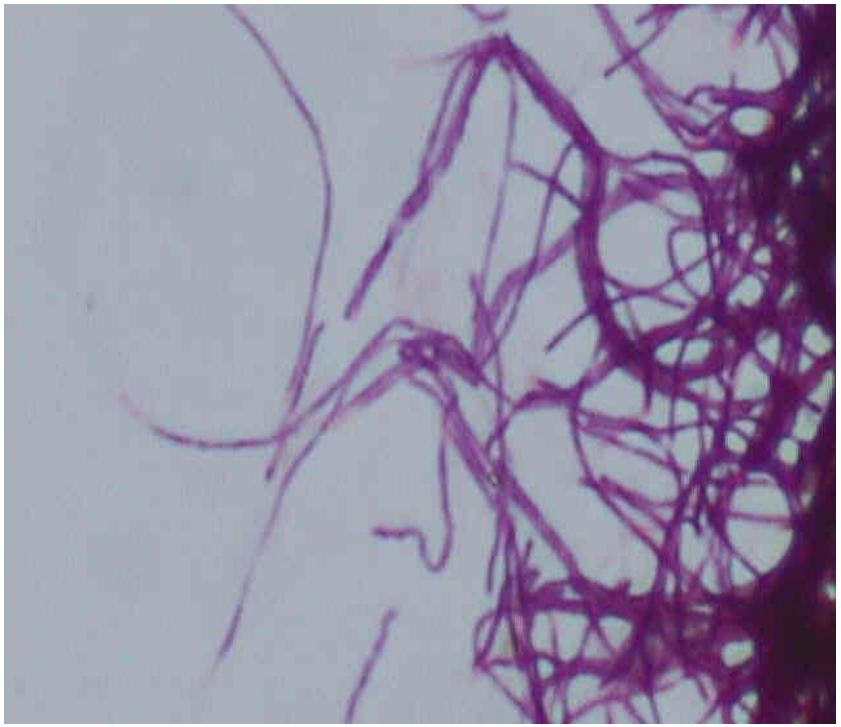


# Scheme for Streptomyces stained by Gram stain

- **Type of stain**: Differential stain (Gram stain).
- **Name of dye**: Crystal violet (1ry stain), Iodine (mordant), Ethanol 95% (decolorizer) and Safranin (counter stain)
- **Color**: Gram positive stained with deep violet
- **Culture:** single.
- **Shape**: Threads.

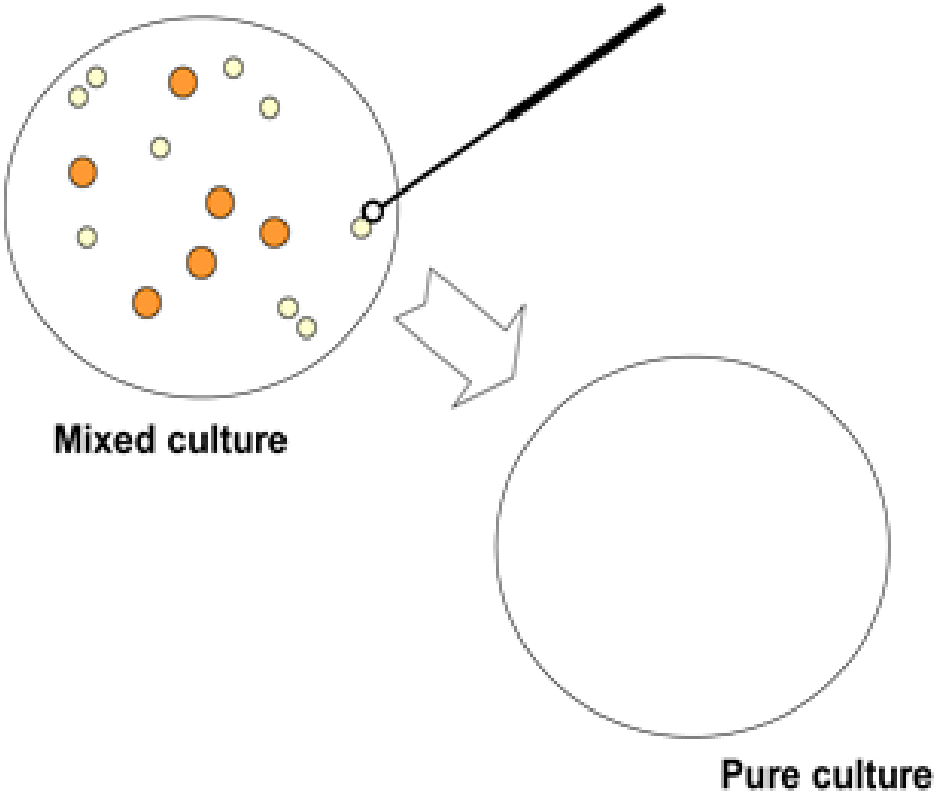
- **Comment**

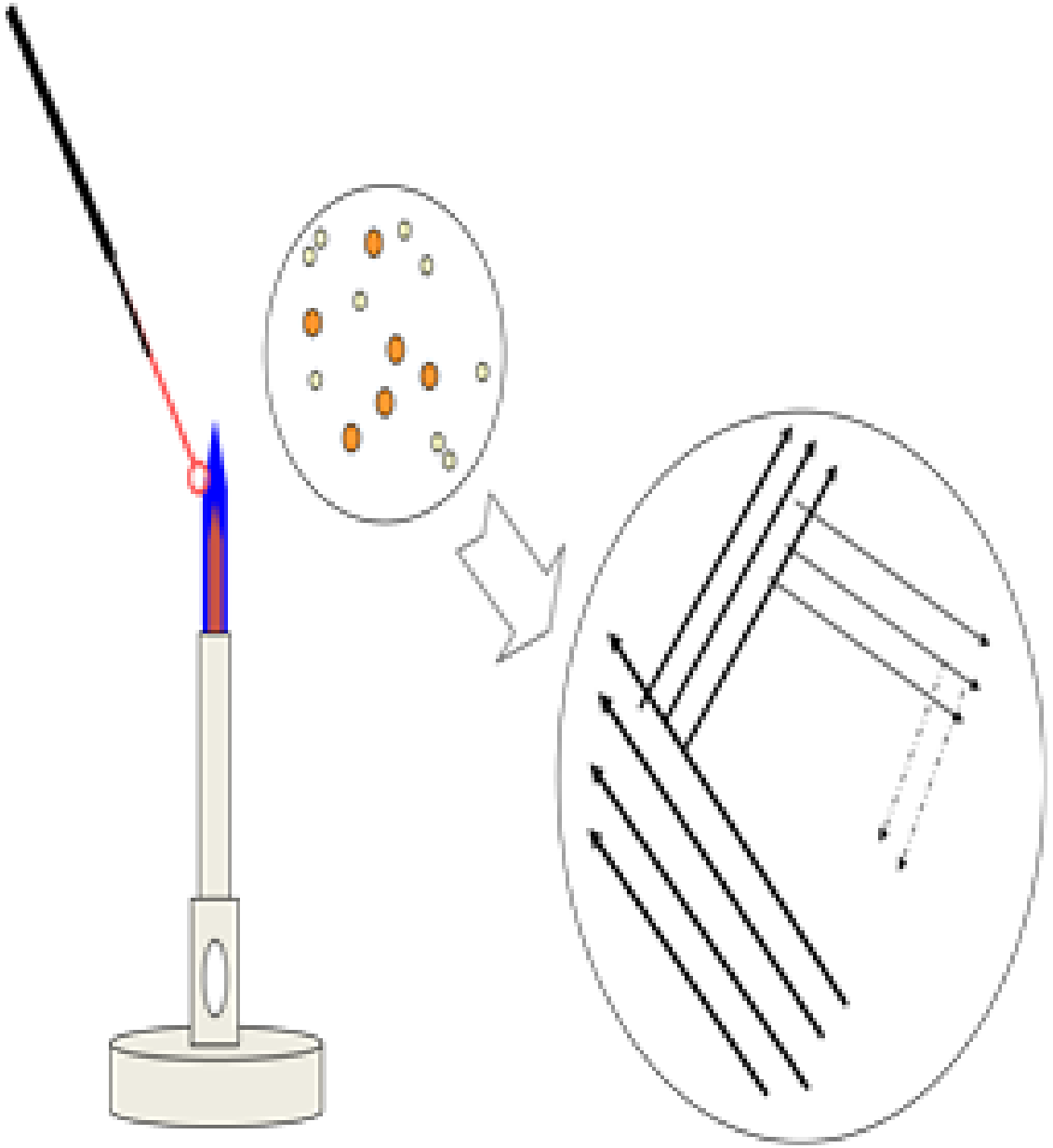
- In Gram **positive** bacteria, cell wall contain **low** lipid content.
- 95% ethanol dissolves lipids , leaving **small** pores **not allowing** the escape of **(Iodine-crystal violet complex)**.
- So the bacteria retain the deep violet colour.

- In Gram **negative** bacteria, cell wall contain **high** lipid content.
- 95% ethanol dissolves lipids , leaving **large** pores **allowing** the escape of **(Iodine-crystal violet complex)**.
- So when saffranin is added ,the bacteria stained with red colour.

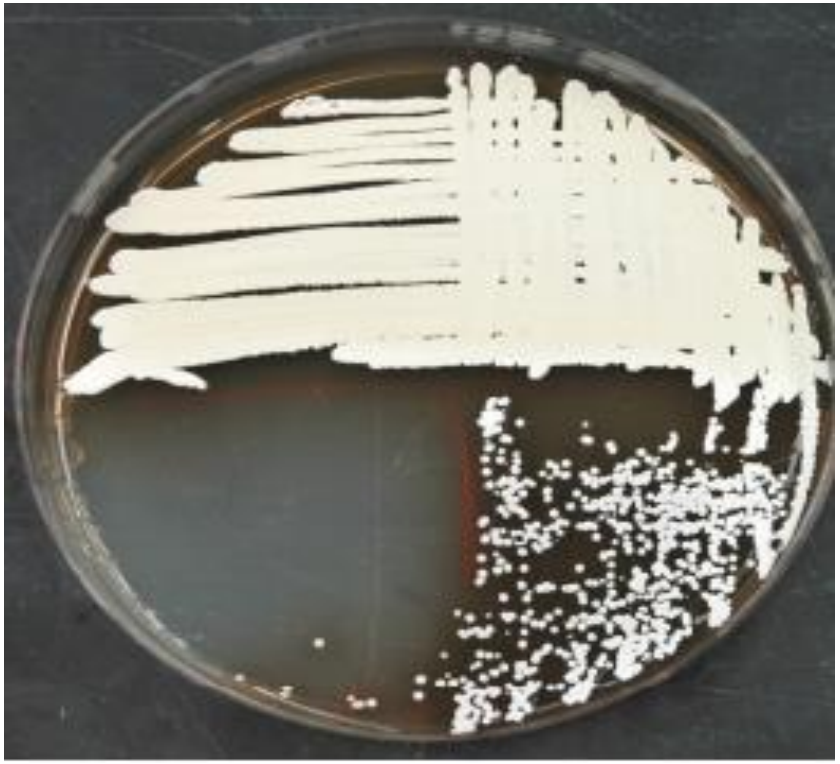
# 3- Streaking for isolation

- With an inoculating loop streak *Streptomyces* colony on the transfer media for isolation of pure colonies.
- Incubate for 3-5 days at room temp (at dark)


Mixed culture
Pure culture



# After incubation




Thank
you